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14. ABSTRACT

This report provides a summary of the progress established by our multidisciplinary, highly synergistic team of researchers from M.D. Anderson Cancer Center and Rice University on their continuous effort of exploring and development of nanoparticle-based methods to breast cancer detection, imaging, and therapy. During the first two years of this project, the team has demonstrated in a series of controlled experiments that the intense local heating induced by infrared illumination of nanoshells can be used to photothermally ablate cancer cells tagged with nanoshells. One of the recent accomplishments of the team was the development of a novel nanoshell-based all-optical platform technology for integrating cancer imaging and therapy. Nanoshells, designed to both absorb and scatter in the NIR spectral region, were conjugated with anti-HER2 antibody to specifically bind to SKBr3 breast cancer cells. Upon binding to the cells, nanoshells targeted against HER2 were seen to provide a significant increase in the scatter-based optical contrast. The anti-HER2-labeled nanoshells alone were seen to be nontoxic to the cancer cells. However, after irradiation with NIR light, the conjugated nanoshells were seen to mediate photothermal effects that resulted in the death of cancer cells. These studies indicate that immunotargeted nanoshells can provide, in a single nanoshell, scattering contrast for imaging cancer cells with high sensitivity, while also exhibiting sufficient absorption to enable effective photothermal therapy of the cancer cells.

15. SUBJECT TERMS
Nanoshells, cancer therapy, cancer imaging, nanoparticle

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Introduction

Over the past year, our multidisciplinary, highly synergistic team of researchers from M.D. Anderson Cancer Center and Rice University has continued exploring and development of nanoparticle-based methods to breast cancer detection, imaging, and therapy. Based on the innovative, optically active gold nanoshells, first developed in the Halas Nanoengineering Group at Rice University,[1] the appealing optical properties of nanoshells have been exploited to achieve the goals proposed in the original proposal statement. Nanoshells, which consist of a dielectric core and a gold shell, posses unique optical properties determined by their core-shell ratio.[1-2] The size can be designed to provide the nanoshells with unique optical absorption and scattering capabilities in the NIR "optical window", a region of the spectrum where physiological transmissivity is highest, making them suitable for bioimaging and biosensing applications. During the first two years of this project, the team has demonstrated in a series of controlled experiments that nanoshells with strong absorption in the near-infrared region can be used to mediate photothermal ablation of cancer cells by inducing intense local heating into the surrounding matrix.[3-4] In contrast with these NIR-absorbing therapeutic nanoshells, the team has also demonstrated, for the first time, that highly scattering NIR nanoshells can be used as high imaging contrast agents which can be coupled to cancer therapy applications.[5] In this report, we provide a summary of the team efforts and accomplishments over the last year of this DOD CDMRP project, including established progress since the starting date of the project. During the past year, the team has dedicated tremendous effort on demonstrating that immunotargeted nanoshells can provide high scattering contrast for imaging while exhibiting sufficient absorption to enable effective photothermal therapy of cancer.

Body

During the past year, our multidisciplinary team has continued to address the following main goals, introduced in the original proposed work:

1- Bioconjugation-based Targeting of Nanoparticles for Imaging and Therapy
2- Nanoparticle-based Image Enhancement
3- Molecular Fingerprinting
4- Nanoshell-based Photothermal Cancer Therapy

Project 1: Bioconjugation-based Targeting of Nanoparticles for Imaging and Therapy

Aim 1: Identification of the optimal ligand-receptor pair for vascular targeted therapy in breast cancer.

Accomplishments (months 1-48): Human epidermal growth factor receptor 2 (HER2) has been identified as a proven molecular marker of breast cancer cells (SKBr3). Nanoshells labeled with HER2 have been prepared and were shown to successfully target the SKBr3 cancer cells.

Aim 2: Development of bioconjugate chemistry for binding targeting peptides and antibodies to nanoshells and nanoemitters.

Accomplishments (months 1-48): Both the specific HER2 antibody and the non-specific IgG antibody have been successfully tethered to the surface of nanoshells. An optimized protocol was established for biding these antibodies to the surface of nanoshells. The antibodies were first attached to a polyethyleneglycol (PEG) linker [orthopyridyldisulfide-polyethyleneglycol-N-hydroxysuccinimide (OPSS-PEG-NHS)] through a hydroxysuccinimide group. The antibody-PEG linker complex was then attached to the nanoshell surface through a sulfur-containing group located at the distal end of the PEG linker. After antibody conjugation, the surface of the nanoshells was coated with PEG-thiol (MW 5000) in order to block non-specific adsorption sites and to enhance biocompatibility.[3,5]

Project 2: Nanoparticle-based Image Enhancement

Aim 1: Investigation of the effectiveness of gold nanoshells and rare earth nanoemitters as image enhancers in tomographic infrared imaging (months 12-48).

Accomplishments: Limited work has been done on Aim 1.

Aim 2: Evaluation of the improvements in image contrast and resolution due to nanoshell-based and nanoemitter-based contrast enhancement.

Accomplishments (12-48): Gold nanoshell bioconjugates for optical imaging have been fabricated. The size of the nanoshells was controlled to provide the nanoshells with unique optical scattering capabilities in the NIR region of the spectrum, allowing optical imaging both in the visible and the NIR regions (Figure 1). The targeted nanoshell bioconjugates were utilized as high contrast agents to image human epidermal growth factor receptor 2 (HER2) expression in living human breast carcinoma cells.[6] Our studies indicated that under dark-field microscopy, a significant increase in the imaging optical contrast was observed when HER2-positive SKBr3 breast cancer cells were targeted with anti-HER2-labeled nanoshells compared with cells targeted by either anti-IgG-labeled nanoshells (non-specific targeting) or cells not exposed to nanoshells bioconjugates (Figure 2). The average imaging contrast values in cells targeted by anti-HER2-labeled nanoshells was significantly ($p<0.05$) greater (142±16) than in cells targeted by anti-IgG (48±12) or cells not exposed to nanoshell bioconjugates (26±4) (Figure 3). Images of HER2-negative MCF7 cells exposed to anti-HER2-labeled nanoshell bioconjugates showed significantly lower contrast (34±5) compared to HER2-positive SKBr3 cells (142±16). This provides additional evidence of contrast enhancement attributed to nanoshells targeting HER2 receptors.[6] More details are found in the attached journal articles No 1.

Aim 3: Demonstration of the feasibility of targeted nanoshell-based imaging and nanoemitter-based imaging in a mouse tumor model.

Accomplishments (months 12-48): Work on Aim 3 is in progress.

Project 3: Molecular Fingerprinting

Aim 1: Examining the feasibility of noninvasive "molecular fingerprinting" in a tissue culture model.

Accomplishments (12-48): Using a novel bi-functional linker constructed of para-mercaptoanaline-poly(ethyleneglycol)-fluorescein (pMA-PEG-Fluor), and utilizing two independent techniques, fluorescence spectroscopy and surface enhanced Raman spectroscopy (SERS), our group has been able to quantitatively determine the number of pMA-PEG-Fluor molecules required to form a complete self-assembled monolayer (SAM) on the surface of gold nanoshells (scheme 1). Fluorescence spectroscopy was used to provide quantitative measurements of the number of PEG-chains on the nanoshell surface. After self-assembly of the pMA-PEG-Fluor molecules on the nanoshell surface, a solution of 0.2 M KCN and 2 mM $K_3Fe(CN)_6$ in water was used to dissolve the nanoshells. Fluorescence measurements were then performed on the resulting supernatant and the measured fluorescence intensity at 519 nm was then used to determine the number of pMA-PEG-Fluor molecules. Surface enhanced Raman spectroscopy of pMA molecule was used to determine the surface coverage at various concentrations following the relationship for a Langmuir isotherm (figure 4).[7] Our studies indicate that both fluorescence spectroscopy and Raman spectroscopy can be correlated to obtain an effective strategy for obtaining quantitative information of SAMs on nanoshells. This holds promising future for designing efficient nanoshell bioconjugates for biological analysis and chemical identification with high sensitivity. Such nanoshells can be used for enhanced cancer detection and therapy and molecular fingerprinting applications.

Aim 2: Investigation of spectroscopic detection of early lesions.

Accomplishments (12-48): Work on Aim 2 is in progress.

Project 4: Nanoshell-based Photothermal Cancer Therapy

Aim 1: Evaluation of the therapeutic effect of targeted nanoshells in animal models of breast cancer.

Accomplishments (1-48): *In vivo* localized, irreversible photothermal ablation of tumor tissue have been demonstrated using nanoshells designed to absorb near infrared light.[3] Nanoshells were injected interstially into solid tumors in female SCID mice. The injected nanoshells accumulated on the tumor sites were seen to induce no cell death until these sites were exposed to NIR light, where then localized cell death occurred. Temperatures were monitored via phase-sensitive, phase-spoiled gradient-echo MRI. Magnetic resonance temperature imaging (MRTI) demonstrated that tumor reached temperatures which caused irreversible tumor damage ($\Delta T = 37.4 \pm 6.6$ °C) within 4-6 minutes. Controls which were exposed to a saline injection instead of nanoshell injection experienced significantly reduced average temperatures after exposure to the same dose of NIR light ($\Delta T < 10$ °C).[3]

Aim 2: Development of a strategy for combined imaging and therapy.

Accomplishments (12-48): A novel nanoshell-based all-optical platform technology for integrating cancer imaging and therapy has been developed. In a recent publication [attached journal article No 2], the team has demonstrated that immunotargeted nanoshells can provide scattering contrast for imaging while also exhibiting sufficient absorption to enable effective photothermal therapy.[8] Nanoshells, designed to both absorb and scatter in the NIR spectral region were conjugated with anti-HER2 antibody to specifically bind to SKBr3 breast cancer cells. Upon binding to the cells, nanoshells targeted against HER2 were seen to provide a significant increase in the scatter-based optical contrast compared to cells targeted by either no nanoshells or nanoshells conjugated with the non-specific antibody (anti-IgG) (Figure 5). The anti-HER2-labeled nanoshells alone were seen to be nontoxic to the cancer cells. However, after irradiation with NIR light, the conjugated nanoshells were seen to mediate photo-thermal effects that resulted in the death of the cancer cells (Figure 5). This effect was not observed in cells exposed to either nanoshells conjugated with the non-specific antibody (anti-IgG) or NIR alone (Figure 5). Greater silver staining intensity was seen in cells exposed to anti-HER2-labeled nanoshells as opposed to controls, indicating enhanced binding of the targeted nanoshells to cell surfaces expressing HER2.[8]

Key Research Accomplishments

- Nanoshells labeled with human epidermal growth factor receptor 2 (HER2) have been prepared and were shown to successfully target SKBR3 cancer cells.
- Anti-HER2-labeled nanoshells have demonstrated a significantly increased optical contrast for imaging cancer cells.
- Localized, irreversible photothermal ablation of tumor tissue in mice have been demonstrated using nanoshells designed to absorb near infrared light.
- Nanoshells targeted against a clinically relevant biomarker HER2 were demonstrated for the first time as both imaging and therapeutic agents of cancer.

Reportable Outcomes

Published

Papers

Christopher Loo, Leon Hirsch, Min-Ho Lee, Emmanuel Chang, Jennifer West, Naomi Halas, Rebekah Drezek "Gold nanoshell bioconjugates for molecular imaging in living cells," *Optics Letters*, 30 (9): 1012-1014 (2005).

Christopher Loo, Amanda Lowery, Naomi J. Halas, Jennifer L. West, Rebekah Drezek "Immunotargeted Nanoshells for Integrated Cancer Imaging and Therapy," *Nano Letters*, 5(4): 709-711 (2005).

Alex W. Lin, Christopher H. Loo, Leon R. Hirsch, Jennifer K. Barton, Min-Ho Lee, Naomi J. Halas, Jennifer L. West, Rebekah A. Drezek "Nanoshells for Integrated Diagnosis and Therapy of Cancer," *Proceedings of SPIE*, 5593: 308-316 (2004).

Christopher Loo, Alex Lin, Leon Hirsch, Min Lee, Jennifer Barton, Naomi Halas, Jennifer West, Rebekah Drezek "Nanoshell-Enabled Photonics-Based Imaging and Therapy of Cancer," *Technology in Cancer Research and Treatment*, 3(1): 33-40 (2004).

D. Patrick O'Neal, Leon R. Hirsch, Naomi J. Halas, J. Donald Payne, Jennifer L. West "Photo-Thermal Tumor Ablation in Mice Using Near Infrared-Absorbing Nanoparticles," *Cancer Letters*, 209(2): 171-176 (2004).

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J. Chen, A. Lin, C. Loo, K. Hsu, J. West, N. Halas, and R. Drezek "Optical Technologies for Noninvasive Functional and Molecular Imaging," in *Advanced Therapy of Breast Disease*, edited by S. Singletary, G. Robb, and G. Hortobagyi, BC Decker: 859-868 (2004).

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In Press

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Halas, N., O'Neal, P., Watkins, D., Halas, N., Drezek, R. and West, J., "Near Infrared Laser Tissue Welding Using Nanoshells as an Exogenous Absorber" Lasers in Surgery and Medicine. In press (2005).

O'Neal, D. P; Hirsch, L. R.; Halas, N. J., Payne, J. D., West, J. L. "Photothermal Cancer Therapy Using Intravenously Injected Near-Infrared Absorbing Nanoparticles," *Proceedings of SPIE*, in press, 2005.

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L. Hirsch, A. Lowery, R. Drezek, N. Halas, and J. West, "Biomedical Applications of Nanoshells," in *Encyclopedia of BioMEMs and Bionanotechnology*, edited by Tejal Desai and Sangeeta Bhatia, Kluwer, in press (2005).

Papers In Review

E. Chang, M. Wargo, J. Chen, A. Lin, C. Loo, and R. Drezek "Optical Imaging of Cancer," *Critical Reviews in Biomedical Engineering*, in review (2005).

A. Lin, N. Lewinski, J. West, N. Halas, and R. Drezek "Computational Analysis of Nanoshells as Optical Contrast Agents for Early Precancer Detection," *Journal of Biomedical Optics*, in review (2005).

Lee, M., Agrawal, A., West, J., Halas, N., and Drezek, R. "Optimized Nanoshells for 1300 nm Optical Coherence Tomography" *Optics Letters*, in review (2005).

Conference Proceedings

Lowery, A.; O'Neal, P.; Loo, C.; Hirsch, L. R.; Stafford, J.; Hazle, J.; Halas, N. J.; Drezek, R.; West, J. L. "Photothermal Tumor Therapy with Metallic Nanoshells," World Biomaterials Congress, Sydney, Australia, May 2004.

Gobin, A. M.; O'Neal, P.; Halas, N. J.; Drezek, R.; West, J. L. "Nanoshells as Near Infrared Absorbers to Enhance Laser Tissue Welding," World Biomaterials Congress, Sydney, Australia, May 2004.

Loo, C.; Hirsch, L.; Halas, N.; West, J.; and Drezek, R. "Towards Molecular Imaging Using Gold Nanoshells," World Biomaterials Congress, Sydney, Australia, May 2004.
Loo, C.; Lowery, A.; West, J. L.; Halas, N. J.; Drezek, R. "Molecular Imaging of Breast Cancer using Gold Nanoshells," World Biomaterials Congress, Sydney, Australia, May 2004.

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Lee, M.; West, J.; Halas, N.; and Drezek, R. "Nanoengineered Optical Contrast Agents," NanoSummit. Houston, TX, July 2004.

Halas, N. J. "Nanoshells: Optimizing Nanophotonic Properties for Probing Living Systems," Abstracts of papers, 229th ACS National Meeting. San Diego, CA, March 2005.

Agrawal, A.; Huang, S.; Pfefer, P.; Lee, M.; and Drezek, R. "Quantitative Evaluation of Nanoshells as a Contrast Agent for 1300 nm OCT," Conference on Lasers and Electro-Optics (CLEO). Baltimore, MD, 2005.

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Presentations

Optical Imaging for Minimally Invasive Medical Diagnosis. Department seminar. Princeton University. April 2005.

Nanoshell Bioconjugates in Cancer Imaging. MD Anderson Grand Rounds. Houston, TX. April 2005.

Nanoshells for Imaging. University of Arizona. Department of Biomedical Engineering Seminar Series. Tucson, AZ. December 2004.

Diagnostic Applications of Nanoshells. Optics East. Bionanotechnology Symposium. Philadelphia, PA. October 2004.

Sensing Cancer Specific Signatures Using Nanoshell Bioconjugates. Rice-UK Conference on Sensing and Imaging Using Nanomaterials. October 2004.

Nanoparticle-Enabled Optical Molecular Imaging. NCI Symposium on Imaging and Advanced Technologies. Washington DC. October 2004.

Nanoshell Cancer Imaging and Therapy. NCI Scientific Advisory Board Meeting. Washington, DC. July 2004.

Towards Nanotechnology-Enabled Breast Cancer Imaging. NanoSummit 2004. Houston, TX. July 2004.

Nanotechnology in Cancer Imaging. Fifth Annual CAMD Workshop: Nanotechnology in Bioscience, Biotechnology, and Medicine. New Orleans, LA. June 2004.

Nanotechnology Academy for High School AP/IB and Honors Teachers. Faculty speaker on nanoshells in medicine. Summer 2004.

Rice University YES College Preparatory Intern Program. Faculty speaker on nanoshells. Summer 2004.

Related Invited Talks

"Nanoshells: using Nanotechnology to harvest light for Biomedicine," Benson Lecturer, Physics Department, Miami University, April 2004. Prof. Naomi Halas, Ph.D

"Nanoshells: applications of Plasmonic Nanostructures in Biomedicine," NIH/NIAID Workshop, Gaithersburg, MD, June 2004. Naomi Halas, Ph.D.

"Nanoshells: from plasmon physics to cancer therapy," Research Seminar, Chalmers University, Goteborg, Sweden, June 2004. Naomi Halas, Ph.D.

"Nanotechnology in Cancer Imaging," Fifth Annual CAMD Workshop: Nanotechnology in Bioscience, Biotechnology, and Medicine. New Orleans, LA. June 2004. Rebekah Drezek, Ph.D.

"Towards Nanotechnology-Enabled Breast Cancer Imaging," NanoSummit 2004. Houston, TX. July 2004. Rebekah Drezek, Ph.D.

"Truth and Beauty at the Nanoscale: Texas-Sized Molecules and Cancer Therapy," Houston Philosophical Society, September 2004. Naomi Halas, Ph.D.

"Breast Imaging Applications of Nanoshell Bioconjugates," Optics East. Bionanotechnology Symposium. Philadelphia, PA. October 2004. Rebekah Drezek, Ph.D.

"Nanoshell-Enabled Optical Molecular Imaging," NCI Symposium on Imaging and Advanced Technologies. Washington DC. October 2004. Rebekah Drezek, Ph.D.

"Nanoshells in Biomedical Applications," U. T. M. D. Anderson Imaging Physics Research Seminar, Houston, TX, November 2004. Naomi Halas, Ph.D.

"Nanoshells for Breast Cancer Imaging," SPRING 2004. Dallas, TX. November 2004. Rebekah Drezek, Ph.D.

"Nanoshells: manipulating light at nanoscale dimensions for biomedicine," University of Texas Center for Biomedical Engineering Annual Conference, Westin, Galleria, Houston, TX, December 2004. Naomi Halas, Ph.D.

"Nanoshells in Biomedicine," AIChE Regional Meeting, Beaumont, TX, February 2005. Naomi Halas, Ph.D.

Continuing Education Courses

Rice Alumni College. Nanotechnology in Medicine lecture, Spring 2005.

The Year's Best: Nanotechnology. Rice Teaching Award Winners Continuing Studies. Lecture Series, Spring 2005.

Nanotechnology-Enabled Medical Imaging. School of Continuing Studies. Nanoscience: The Science of the Very Small. Lecture Series, Fall 2004.

Nanotechnology in Imaging. School of Continuing Studies Nanotechnology Academy. Rice University. Houston, TX, July 2004.

Patents:

West, J., Drezek, R, Sershen, S., and Halas, N. "Optically-absorbing Nanoparticles for Enhanced Tissue Healing." U.S Patent No. 6,685,730.

Conclusions

There is a continuous need for the development of novel materials and innovative technologies to significantly impact the future strategies towards cancer detection, diagnosis, and therapy. Our team has developed a number of clinical tools for diagnosis and therapy of breast cancer based on the innovative, optically active gold nanoshells. Nanoshell-based molecular contrast agents offer unique advantages including NIR-tunability, size flexibility, non-cytotoxicity, and systematic control of optical scattering and absorption properties. Our studies have shown that nanoshell bioconjugates can be used to target specific cancer markers, allowing *in vitro*, high contrast molecular optical imaging on a cellular level, and at the same time exhibit sufficient absorption to enable effective photothermal therapy of the cancer cells. This demonstrates that in a single technology, and based on nanoshells, both diagnostic and therapeutic capabilities can be achieved. This would yield in a significant savings in time, cost, and patient discomfort associated with diagnosing and treating cancer today. Current results encourage future work assessing nanoshell as imaging and therapeutic agents for in vivo studies utilizing

sophisticated techniques such as reflectance confocal microscopy (RCM) and optical coherence tomography (OCT). The combination of targeted optical contrast agents and photonics imaging technologies has the potential to play a vital role in the future of cancer screening and diagnosis, in designing and monitoring therapeutic interventions, and in fundamental studies of carcinogenesis.

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6. Loo, C.; Hirsch, L.; Lee, M.; Chang, E.; West, J.; Halas, N.; Drezek, R. "Gold Nanoshell Bioconjugates for Molecular Imaging in Living Cells," *Optics Letters*, 30 (9), 1012-1014 (2005).

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8. Christopher Loo, Amanda Lowery, Naomi J. Halas, Jennifer L. West, Rebekah Drezek "Immunotargeted Nanoshells for Integrated Cancer Imaging and Therapy," *Nano Letters*, 5(4), 709-711 (2005).

Appendices

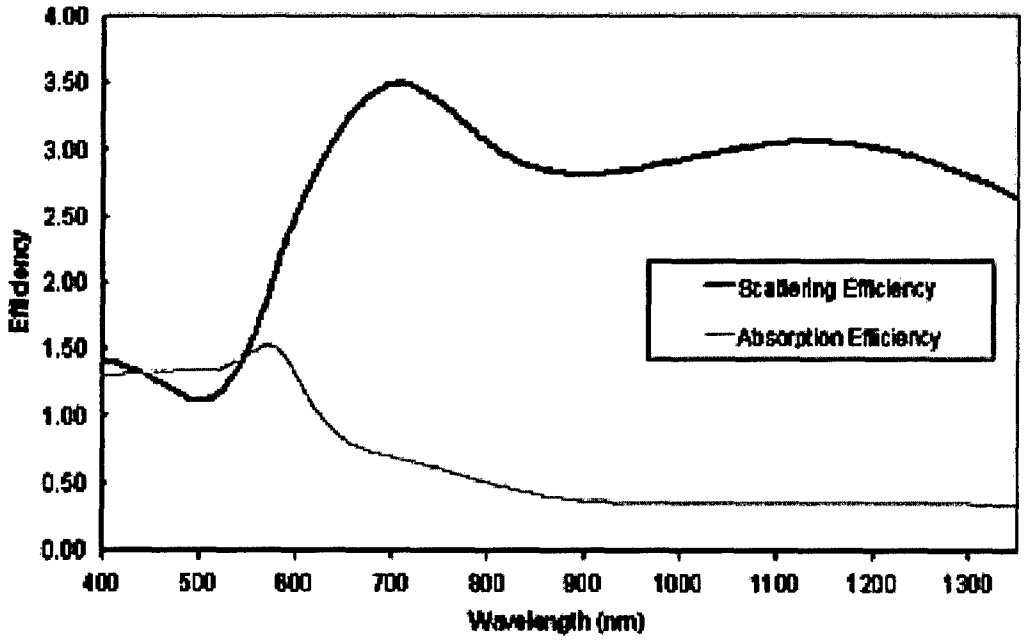


Figure 1. Scattering (black) and absorption (gray) efficiencies predicted by Mie theory for nanoshells with 120-nm silica core radius and 35-nm shell thickness that were used as contrast agents for cancer imaging.

Figure 2. (A)-(C) High magnification dark-field images of HER2-positive SKBr3 breast cancer cells exposed to: no nanoshell bioconjugates (A&D), anti-IgG-labeled nanoshell bioconjugates (B&E), or anti-HER2-labeled nanoshell bioconjugates (C&F). Images (D) through (F) illustrate nanoshell targeting and coating of the cell surface at lower magnification.

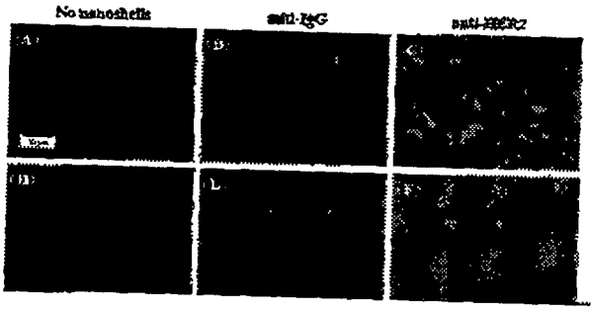
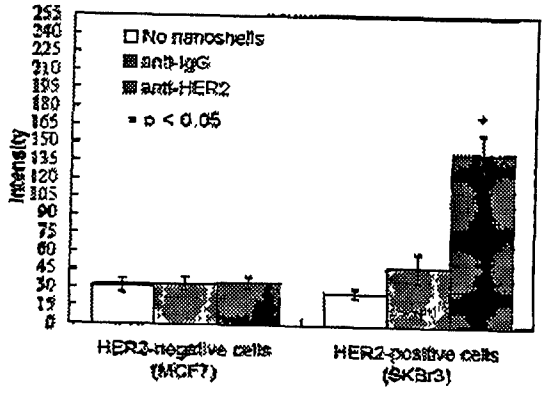
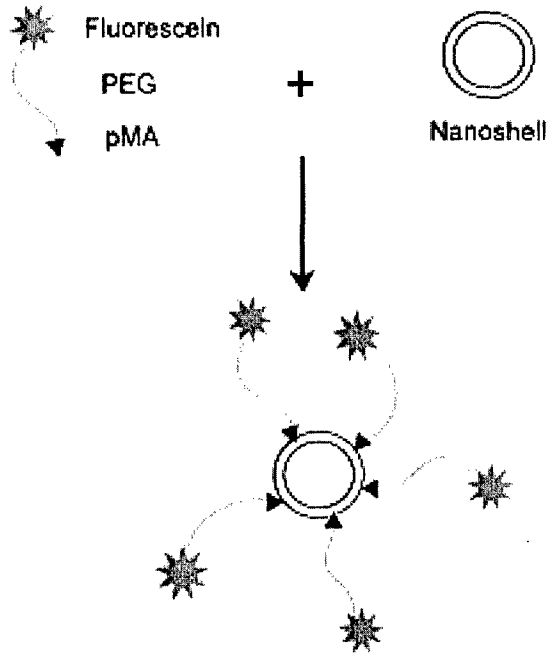


Figure 3. Histogram of optical contrast analysis illustrating the measured scattering intensity in different groups of HER2-positive SKBr3 cells compared to different groups of HER2-negative MCF7 cells. Contrast was quantified by obtaining average histogram intensity values of dark-field images. Intensity values range from 0 (black) to 255 (white), with higher values corresponding to greater contrast. Differences in mean scattering intensity between the anti-HER2 group and all the other cell groups were statistically significant ($p<0.05$).





Scheme 1. Self-assembly of pMA-PEG-fluorescein on a gold nanoshell.

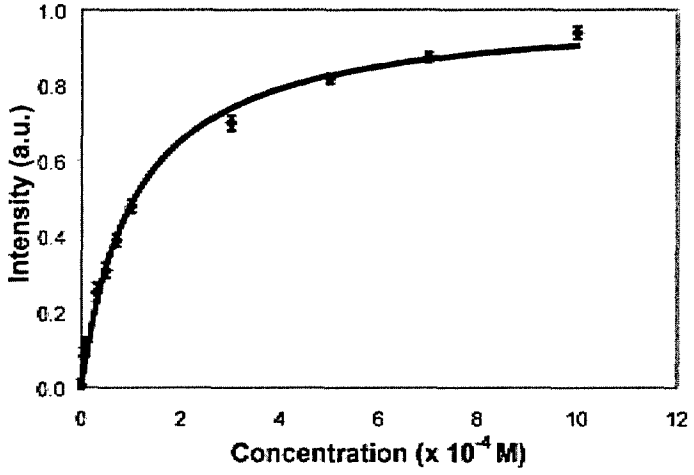


Figure 4. SERS intensity at various pMA concentrations on gold nanoshells (circles) and Langmuir Isotherm (line). The shown intensity represents the intensity of the ring breathing mode of pMA at 1080 cm^{-1}.

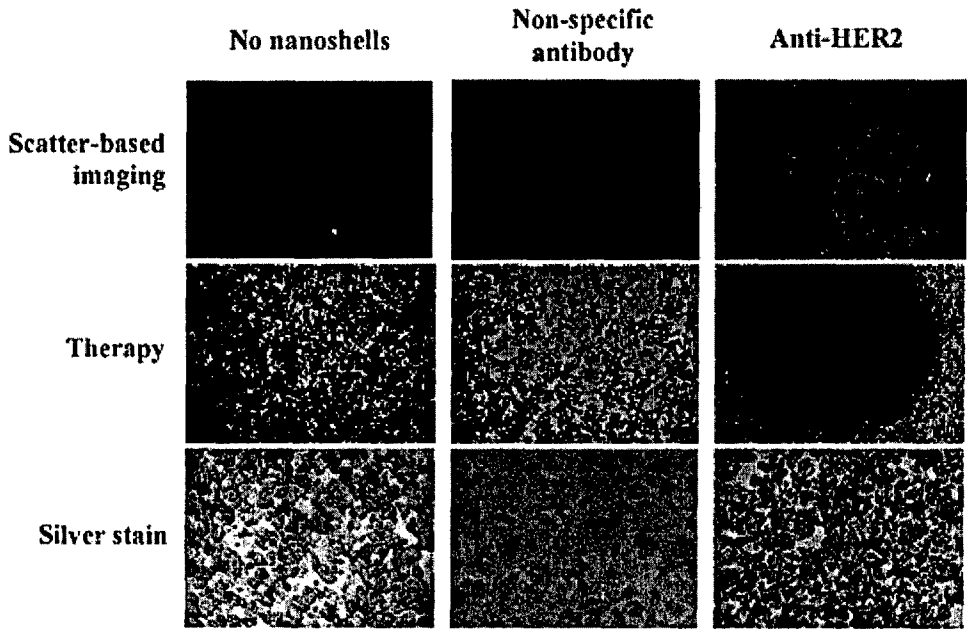


Figure 5. Combined imaging and therapy of SKBr3 breast cancer cells using HER2-targeted nanoshells. Top row represents scatter-based dark-field images of cells exposed to: no nanoshells (left column), non-specific anti-IgG-labeled nanoshells (middle column), and anti-HER2-labeled nanoshells (right column). Middle row shows images of cells viability assessed via calcein staining. Cytotoxicity was observed in cells targeted with anti-HER2-labeled nanoshells (middle row, right column) after exposure to NIR-emitting laser. Enhanced imaging contrast (top row, right column) and cytotoxicity (middle row, right column) are noted in cells targeted by anti-HER2-labeled nanoshells and after exposure to NIR light as compared to controls (left and middle columns). Bottom row shows images of silver stain assessment of nanoshell binding.